protein Von Willebrand factor (VWF), however, under specific conditions WPBs also contain a cocktail of small pro-inflammatory cytokines. WPB exocytosis is driven by an increase in intracellular free calcium ion concentration ($[Ca^{2+}]_i$); the majority of WPB fusion events result in complete discharge of cargo components, however, in a small fraction of cases cytokines are selectively released indicating that the fusion pore may act as a molecular size filter. Carbon fibre amperometry has been used to characterize fusion pore behavior in a number of cell types, but to date this approach has not been applied to WPBs. Therefore, we used this technique in combination with simultaneous optical imaging of fluorescent WPB exocytosis and changes in $[Ca^{2+}]_i$ and can report, for the first time, the kinetic properties of WPB fusion pore formation and expansion in human cultured endothelial cells. A clear delay (mean ~50 ms) is seen between the onset of the current spike and the increase in intra-WPB EGFP fluorescence indicating that WPB alkalinsation is delayed by the strong proton buffering capacity of the WPB lumen (55 mM/pH unit). Analysis of current spike parameters reveal a mean 25-75% rise time, peak amplitude and decay time of 1.63 ms, 50 pA and 6.63 ms respectively. Approximately 50% of current spikes were preceded by a foot signal of mean duration 6.34 ms. Occasional low amplitude, prolonged current increases, reminiscent of stand alone foot signals, were observed in conjunction with morphological rounding of WPBs, possibly reflecting kiss-and-run or lingering kiss fusion events. Following characterization of the WPB fusion pore under control conditions the impact of changing cellular parameters, including cholesterol levels, was also assessed.

1611-Pos Board B381

Role of the Cytoskeleton in the Regulation of Weibel-Palade Body Exocytosis

Nicola Hellen[1], Ruben Bierings[1], Laura Knipe[1], Gregor Zupancic[2], Matthew J. Hannah[3], Tom Carter[1].
[1]National Institute for Medical Research, London, United Kingdom, [2]University of Ljubljana, Ljubljana, Slovenia, [3]Health Protection Agency, London, United Kingdom.

Calcium-driven secretion of the haemostatic protein Von Willebrand factor (VWF) is mediated through exocytosis of specialized endothelial cell (EC)-specific secretory organelles called Weibel-Palade bodies (WPBs). Biochemical studies indicate that VWF secretion is regulated by the cytoskeleton and that the Rab27a-MyRIP-myosinVa complex plays a role in this process, however, little is known about their contribution to the underlying kinetics and extent of WPB exocytosis. To address this we have combined biochemical approaches with simultaneous high speed imaging of intracellular free calcium and exocytosis of fluorescent WPBs in living human umbilical vein endothelial cells (HUVEC) stimulated with ionomycin (1μM) or histamine (100μM). Cytochalasin-D disruption of actin had a subtle effect on the kinetics and extent of ionomycin-evoked WPB exocytosis; delays to the first fusion event and maximal rate of WPB exocytosis were not altered. There was a small increase in the extent of fluorescent WPB degranulation, from 61.1 ± 11.5% (± SD, n=19 cells) in control cells to 71.0 ± 15.9% (± SD, n=23 cells) ($p<0.03$), although in separate biochemical experiments a small (10-15%) but non-significant increase in VWF secretion was seen. RNAi knockdown MyRIP resulted in complete loss of WPB associated MyRIP-immunoreactivity and a modest (15-20%) but significant ($P<0.007$) increase in VWF secretion. Disruption of microtubules with Nocodazole substantially decreased (30-40%) VWF secretion and in live cell studies significantly increased the delay and decreased both the rate and extent of ionomycin-evoked WPB exocytosis. Together the data suggests that microtubules play the dominant role in shaping the kinetics and extent of WPB exocytosis, while the actin cytoskeleton and MyRIP play a minor role.

1612-Pos Board B382

Rab27a Regulates Exocytosis of Large Dense-Core Granules in Mouse Chromaffin Cells

Widmann W. Hoerauf, Victor A. Cazares, Arasakumar Subramani, Stephen A. Ernst, Edward L. Stuenkel.
University of Michigan, Ann Arbor, MI, USA.

Rab-GTPases exert important roles in secretory vesicle cycles, with Rab3 and Rab27 participating in exocytotic throughput. While Rab3 and Rab27 share many effectors, some are unique, making it critical to identify specific functional roles. Studies on regulation of neurotransmitter release have focused principally on Rab3 and much less on Rab27. Among Rab-GTPases, Rab27 is unique in binding effectors in either the GTP- or GDP-bound state. We here compare secretory responses from ashen mice, which lack functional Rab27a protein, with strain-matched controls. Secretion was evaluated using measurements of membrane capacitance under whole-cell recording in isolated mouse chromaffin cells. UV flash uncaging of Ca2+ raised [Ca2+]i uniformly and induced catecholamine neurotransmitter release. RT-PCR demonstrated the presence of Rab27 in chromaffin cells, and immunoblotting further characterized the expressed isoform as Rab27a, not Rab27b. The exocytic burst (1s post-UV flash) of membrane capacitance increase (ΔCm) was fitted by a double exponential curve, from which the amount and fusion kinetics of granules from the readily- (RRP) and slowly-releasable pools (SRP) were extracted. Exocytic burst ΔCm was greater, and the size of both the RRP and SRP larger, in wild-type cells (RRP, 347 fF; SRP, 616 fF) than in Rab27a-/- cells (RRP, 157fF; SRP, 438 fF), while the kinetics of release from both pools remained unchanged. Expression of Rab27a in Rab27a-/- cells, using electroporation, rescued the wild-type phenotype. Rab 27a is therefore important for priming into the RRP and SRP. To assess dense-core vesicle docking at the plasma membrane, we analyzed granule distribution from E18 chromaffin cell EM micrographs. These data show that while Rab27a-/- cells possess fewer total granules, morphological docking of granules at the plasma membrane is unaffected. Further work is necessary to identify through which effector Rab27a is exerting its effects.

1613-Pos Board B383

Coordinated Calcium and Cortical Actin Oscillations Regulate a Cellular Secretion Cycle

Roy Wollman, Tobias Meyer.
Stanford University, Stanford, CA, USA.

Exocytosis of secretory granules (SG) is a fundamental biological process that is critical for many physiological functions. Exocytosis requires recruitment of SG and their fusion with the plasma membrane. The actin cortex plays two contradictory roles in SG recruitment: it captures them through Myosin Va motors, bringing them to the periphery of the cell; yet at the same time, it acts as a passive mechanical barrier preventing their approach to the plasma membrane. How do cells consolidate these two opposing roles of the actin cortex? We show, using live-cell multi-color TIRF microscopy, that activation of the FceRI pathway in RBL-2H3 cells results in coordinated oscillation of Ca2+, PIP2, N-WASP, F-actin and Myosin Va. These oscillations alternate between high actin levels that promote SG capture, and low actin levels that reduce the mechanical barrier. This temporal partitioning resolves the conflicting roles of the actin cortex as both a carrier and a barrier of SG.

1614-Pos Board B384

Sub-Frame Time Resolution in Fluorescence Imaging Reveals Delay Between SNAP25 Conformational change and Secretory Events in Chromaffin Cells

Ying Zhao[1], Qinghua Fang[1], Adam Drew Herbst[1], Khajak Berberian[1], Wolfhard Almers[2], Manfred Lindau[1].
[1]Applied and Engineering Physics, Cornell Uinversity, Ithaca, NY, USA, [2]Oregon Health and Sciences University, Portland, OR, USA.

The SNARE-complex is thought to mediate opening of the exocytotic fusion-pore. To determine if a direct relation exists between a conformational-change in the SNARE-complex and the fusion-event, we combine Total-Internal-Reflection-(TIR)-Fluorescence-Resonce-Energy-Transfer-(FRET) imaging with rapid spatially resolved electrochemical detection of fusion-events using ElectroChemical-Detector (ECD) arrays (Hafez-et-al-PNAS-2005-102:13879). The SNARE-Complex-Reporter (SCORE) was used, which is based on the SNARE-protein SNAP25 with FRET-donor CFP and acceptor Venus inserted at the N-termini of its SNARE-motifs SN1&SN2, respectively (An-and-Almers-2004-Science-306:1042). The ECD-array consists of four-platinum-electrode patterned on a glass-coverslip with a space of ~5μm between them where a bovine-chromaffin-cell expressing SCORE was placed such that the FRET changes of SCORE could be imaged by TIR-FRET microscopy, while the ECD-array simultaneously records the exocytotic events as amperometric-spikes. Based on the oxidation currents recorded by the four electrodes, the locations of the exocytotic-events were determined. Averaging the FRET changes at the locations of individual fusion events revealed